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CORRELATION BETWEEN BIOFILM PRODUCTION AND ANTIFUNGAL DRUG RESISTANCE IN CANDIDA SPECIES ISOLATED FROM CASES OF VULVOVAGINAL CANDIDIASIS IN A TERTIARY CARE CENTRE IN SOUTH INDIA.

Shwetha DC[1], Bhavana C[2], Ragini Ananth Kashid[3]

[1]Associate Professor, Department of Microbiology, BGS Global Institute of Medical Sciences, Bengaluru, Karnataka, India.
[2]Assistant Professor, Department of Microbiology, BGS Global Institute of Medical Sciences, Bengaluru, Karnataka, India.
[3] Professor & HOD, Department of Microbiology, BGS Global Institute of Medical Sciences, Bengaluru, Karnataka, India.

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Corresponding Author:
Dr. Ragini Ananth Kashid,
Professor & HOD, Department of Microbiology, BGS Global Institute of Medical Sciences, Bengaluru, Karnataka, India.
Email: ragini.rajgopal@gmail.com

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ABSTRACT

Background: Vulvovaginal candidiasis is a prevalent fungal infection among women of child bearing age group. In recent years, Non-albicans Candida have been isolated more frequently as compared to Candida albicans. The ability of these organisms to form biofilm, contributes towards its pathogenesis and increased resistance to antifungal drugs.

Materials and Methods: High vaginal swabs from suspected cases of vulvovaginitis were subjected to Gram stain and inoculated onto blood agar & MacConkey agar. These culture plates were incubated aerobically at 37°C for 24-48 hours. Speciation of the isolated Candida was done as per standard protocol. Antifungal susceptibility testing was done by the Kirby-Bauer disc diffusion method. Biofilm formation among the Candida isolates were assessed by Microtiter plate method and Visual tube method.

Results: Out of 528 high vaginal swabs that were collected from clinically suspected cases of vulvovaginitis, 115 isolates were identified as Candida spp. Non-albicans Candida accounted for 54.8% of Candida isolates and 45.2% were Candida albicans. The Antifungal susceptibility test results showed that among Candida albicans, 26.9% of isolates were resistant to clotrimazole, 19.2% were resistant to fluconazole, 17.3% were resistant to ketoconazole. Among Non-albicans Candida, 46.1% were resistant to clotrimazole and 17.5% were resistant to fluconazole. No resistance was seen for ketoconazole, amphotericin B and nystatin. In our study, biofilm production was observed in 22 of 52 (42.3%) isolates of Candida albicans, 22 of 32 (68.8%) isolates of Candida tropicalis, 5 of 15 (33.3%) Candida glabrata and 11 of 14 (78.6%) isolates of Candida krusei isolates by Microtiter plate method.

Conclusion: Our study demonstrates shift from Candida albicans to Non-albicans Candida and the association between biofilm formation and antifungal resistance. This study reiterates the need for speciation, antifungal susceptibility testing and detection of biofilm production, as these will help in better understanding of antifungal resistance and clinicians can initiate appropriate treatment for cases of vulvovaginal candidiasis.

Keywords: Antifungal resistance, Biofilm production, Candida albicans, Non-albicans Candida.

INTRODUCTION

Candida species constitute a part of the normal microbial flora of healthy individuals and are considered as opportunistic pathogens capable of colonizing different tissues and cause systemic mycosis when the host immunity is lowered. Over the recent years, the incidence of fungal infections

has progressively raised and it has been a primary cause of morbidity and mortality in immunocompromised and severely ill patients and hence is rightly referred to as "disease of diseased".[1]

Candida exhibits a wide range of clinical manifestations from mucocutaneous lesions to life threatening invasive diseases. Although Candida albicans is the leading cause of candidiasis accounting for about 60-80% of infections, recent epidemiological studies have documented a shift towards Non-albicans Candida infections.[2]

Vulvovaginal candidiasis has been reported to be the second leading cause of vaginitis after bacterial vaginosis. Vulvovaginal candidiasis can cause pain, discomfort, mental distress and anxiety which may in turn affect professional and personal life. It may lead to complications such as pelvic inflammation and abscess, infertility, ectopic pregnancy, abortion or menstrual irregularities if not treated.[3]

Several risk factors have been proposed for vulvovaginal candidiasis. The host related factors include pregnancy, uncontrolled diabetes mellitus, prolonged usage of antibiotics and glucocorticoids, hormone replacement therapy and genetic predisposition. The behavioral factors include oral contraceptives, intrauterine devices, poor hygiene, clothing and sexual practice.[4]

Fluconazole and clotrimazole are the commonly prescribed antifungal drugs, to treat Candida vaginitis. The increasing incidence of antifungal resistance among Candida species, driven by empirical administration or overuse of antifungal drugs poses therapeutic challenge.[5] Evidence from various studies indicate that antifungal resistance is more among Non-albicans Candida when compared to Candida albicans.[6]

Biofilm production is widely recognized as an important virulence factor of Candida species.[7] Biofilms are a group of microorganisms, that are embedded in an extracellular matrix (ECM), forming a complex three-dimensional architecture on biotic and abiotic surfaces.[8] It enables the organism to withstand or evade host defense mechanism, persist as reservoir, serve as a recurrent source of infection and helps in antifungal resistance.[7] Biofilm formation can occur on mucosal surfaces and plastic surfaces of indwelling devices. A majority of the diseases caused by Candida species are associated with biofilm formation.[8]

Candida biofilm imposes serious adverse effects on the health and well-being of patients by impairing the optimum functioning of implant devices, treatment failure resulting in aggravated morbidity and mortality, poor prognosis, prolonged hospital stays, and substantial financial burden to the patient.[7]

This study highlights the need for detection of biofilm production and its association with antifungal resistance among Candida species from vulvovaginal candidiasis in our hospital. This study will provide valuable insights on antifungal resistance, which will enable the clinicians to initiate appropriate and prompt antifungal treatment.

MATERIALS AND METHODS

This prospective, cross-sectional study was conducted over a period of one year (October 2023 to September 2024) at a tertiary care hospital in Bengaluru, South India. The Institutional Ethics Committee approval was obtained for the study. All samples were collected after receiving the informed consent from the patient.

Objectives:

- To isolate and speciate Candida from high vaginal swabs received at the Microbiology laboratory from suspected cases of vulvovaginitis.
- To perform antifungal susceptibility test by Kirby-Bauer disc diffusion method for the isolated Candida species.
- To detect biofilm production by Visual tube method and Microtiter plate method.
- To determine the association between biofilm production and antifungal resistance.

Inclusion criteria

- Patients with clinically suspected vulvovaginitis.
- Candida species isolated from high vaginal swabs received at the Microbiology laboratory.

Exclusion criteria

- Patients on antifungal therapy at the time of sample collection.
- Organisms other than Candida species isolated from high vaginal swabs.

Processing of samples: Two high vaginal swabs were collected from clinically suspected cases of vulvovaginitis (with history of white discharge per vagina, itching, lower abdominal pain) attending the Obstetrics and Gynaecology department of our hospital.

After receiving the samples in the Microbiology laboratory, one swab was used to prepare direct smear and Gram staining was done. The other swab was used to inoculate on to blood agar and MacConkey agar and incubated aerobically at 37°C for 24 hours. If there was no growth after 24 hours of incubation, extended incubation was done for 48 hours. The plates were inspected macroscopically for growth. Any dry, white, pasty looking colonies on blood agar was suspected as Candida and was further identified as per standard protocol (Gram stain, colony morphology on Sabouraud's Dextrose Agar (SDA), CHROM agar, corn meal agar, sugar assimilation and sugar fermentation test).[9,10]

Antifungal susceptibility testing by Kirby-Bauer disc diffusion method: Mueller-Hinton agar (MHA) was prepared by adding 2% glucose and 0.5 µg/ml methylene blue for enhancing the definition of growth margins. Antifungal susceptibility testing was performed by Kirby-Bauer disc diffusion method. The surface of the agar was inoculated by

lawn culture method and the antifungal discs like clotrimazole (10 µg), fluconazole (10 µg), ketoconazole (10 µg), amphotericin B (100 U) and nystatin (100 U) were placed on the surface of the inoculated plate. The results were read after 24-48 hours of aerobic incubation at 37° C. Figure 1 shows results of antifungal susceptibility testing on MHA plate. The diameter of zone of inhibition was measured in millimeters and the results were interpreted as per manufacturer instructions and CLSI guidelines.[11] Candida albicans ATCC 14053 was used as control.

The culture media and antifungal discs were procured from HiMedia laboratories Private Limited, Mumbai, India.

Biofilm formation: Biofilm production among the Candida isolates were detected by the following methods – Microtiter plate method and Visual tube method, as described in Rachel R et al.[12]

Microtiter plate method: The isolated colonies of Candida were added to the test tube containing 5ml of sterile Brain Heart Infusion (BHI) broth, incubated overnight and matched to 0.5 McFarland turbidity standards. 1:100 dilution was done using sterile BHI broth. 100µl of the diluted suspension was added to sterile polystyrene, 96 welled, round bottomed microtiter plate in triplicates and incubated at 37°C for 72 hours. Candida albicans ATCC 14053 was used as a positive control for biofilm production and uninoculated BHI broth was used as negative control.

After 72 hours of incubation, the contents of the plate were removed by gentle tapping and further rinsed three times with PBS (Phosphate Buffered Saline) of pH 7.3 to remove free floating bacteria. 120µl of 0.1% crystal violet was added to stain the plates for 15 minutes at room temperature. Distilled water was used to remove excess stain, blot dried and solubilization of the stain was done by adding ethanol [Figure 2]. The absorbance values were determined using an ELISA reader at 570 nm and the Mean OD was calculated for each isolate.

OD cut off (ODc) was calculated using the formula,

ODc = Mean OD of negative control+ 3 Standard Deviation

Mean OD < ODc = Non biofilm producer

ODc< Mean OD < 2 ODc = Weak biofilm producer

2 ODc< Mean OD < 4 ODc = Moderate biofilm producer

4 ODc< Mean OD = Strong biofilm producer.

Visual tube method: A loopful of overnight Candida culture from Sabouraud's dextrose agar (SDA) was inoculated into 5 ml of sterile Brain Heart Infusion (BHI) broth and incubated at 37°C for 24 hours. The contents of the tubes were decanted and washed thoroughly with PBS. Candida albicans ATCC 14053 was used as a positive control for biofilm production and uninoculated BHI broth was used as negative control. The tubes were completely dried by keeping them in upside down position and stained with 0.1% crystal violet for 15 minutes. The tubes were washed again with distilled water and kept for drying in upside down position. Formation of visible film lining the tubal wall and its bottom indicates biofilm formation. Biofilm production in tubes was classified as weak (+), moderate (++) or strong (+++) as shown in Figure 3.

Statistical analysis

- Data was analyzed by percentage analysis using SPSS version 22 software.
- Chi-square test was used to analyze the correlation between biofilm production and antifungal resistance.

RESULTS

A total of 528 clinically suspected cases of vulvovaginitis cases were included in the present study. 115 Candida species were isolated from these patients and the isolation rate of Candida is 21.8%.

Among 115 Candida isolates, 63 (54.8%) isolates were Non-albicans Candida and 52 (45.2%) were Candida albicans. Candida tropicalis (32, 27.8%) was the predominant isolate among Non-albicans Candida, followed by Candida glabrata (15, 13.1%), Candida krusei (14, 12.2%) and Candida parapsilosis (2, 1.7%). [Figure 4]

The Antifungal susceptibility test results of Non-albicans Candida showed that, out of 32 Candida tropicalis, 21 (65.6%) were resistant to clotrimazole followed by fluconazole resistance seen in 9 (28.1%) isolates. Out of 15 Candida glabrata, 4 (26.7%) and 2 (13.3%) isolates were resistant to clotrimazole and fluconazole respectively. Among 14 Candida krusei, 4 (28.6%) isolates were resistant to clotrimazole. As Candida krusei are intrinsically resistant to azoles, we didn't not test these isolates for fluconazole and ketoconazole. It was noted that, no resistance was observed to ketoconazole among other Non-albicans Candida. Two Candida parapsilosis were isolated in our study and no resistance was seen to the antifungal drugs tested. Among 52 Candida albicans, 14 (26.9%) were resistant to clotrimazole, 10 (19.2%) were resistant to fluconazole and 9 (17.3%) were resistant to ketoconazole. None of the Candida isolates were resistant to amphotericin B and nystatin. Species wise antifungal resistance pattern is depicted in Table 1.

Biofilm detection by Microtiter plate (MTP) method showed that, 60 Candida isolate (52.2%) were biofilm producers and 55 Candida isolates (47.8%) were non-biofilm producers (Figure 5). Among these 60 biofilm producers, 13 (21.7%) Candida isolates were weak biofilm producers, 17 (28.3%) were moderate biofilm producers and 30 (50%) were strong biofilm producers [Figure 6].

Biofilm detection by the Visual Tube Method (VTM) revealed that 55 (47.8%) Candida isolates were biofilm producers and the remaining 60 (52.2%) Candida isolates were non-biofilm producers [Figure 5]. Among the 55 biofilm-producing isolates, 22 (40%) were weak producers,

22 (40%) were moderate producers, and 11 (20%) were strong biofilm producers. [Figure 6].
The biofilm production status of individual Candida species is shown in Table 2.

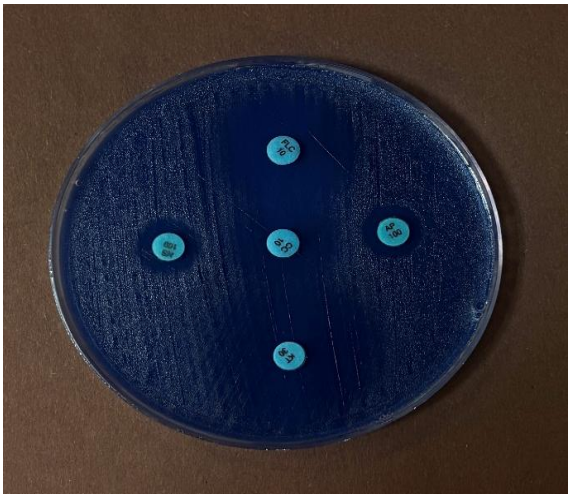

Figure 1: Antifungal susceptibility testing by Kirby Bauer disc diffusion method.

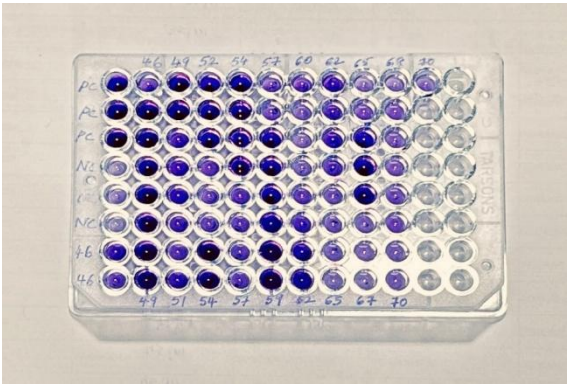

Figure 2: Biofilm detection by Microtiter plate method

PC= Positive Control NC= Negative Control

Figure 3: Biofilm detection by Visual tube method

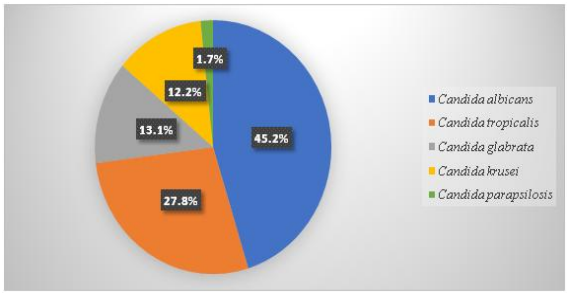

Figure 4: Distribution of Candida species among vulvovaginal candidiasis cases

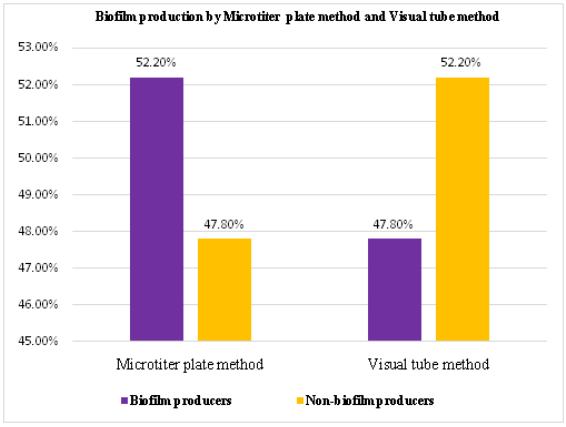

Figure 5: Biofilm production by Candida species detected by Microtiter plate method and Visual tube method

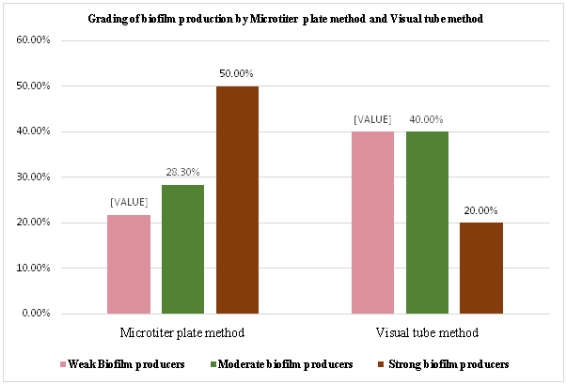

Figure 6: Grading of biofilm production among Candida species detected by Microtiter plate method and Visual tube method

Out of the 52 Candida albicans isolates, 22 (42.3%) were biofilm producers and the remaining 30 (57.7%) were non-biofilm producers. Among the 32 isolates of Candida tropicalis, 22 (68.8%) were biofilm producers and 10 (31.2%) were non-biofilm producers. Of the 15 isolates of Candida glabrata, 5 (33.3%) showed biofilm formation whereas, 10 (66.7%) did not demonstrate evidence of biofilm formation. Among 14 isolates of Candida krusei, 11 (78.6%) were biofilm producers, while 3 (21.4%) were non- biofilm producers. Both isolates of Candida parapsilosis were non-biofilm producers [Table 3].

As per the results of our study, 60 Candida isolates were biofilm producers. We observed that the biofilm production was common among Non-albicans Candida seen in 38 (60.3%) isolates compared to Candida albicans 22 (42.3%) which is depicted in Table 4. The difference in biofilm production between Non-albicans Candida and Candida albicans is not statistically significant (p value= 0.082)

Our study also assessed the correlation between biofilm production and antifungal drug resistance. It was observed that, antifungal resistance was common among biofilm producing Candida isolates as compared to non-biofilm producing isolates which is shown in Table 5. Out of 60 Candida

isolates which are biofilm producers, 45 (75%) showed resistance to one or more antifungal drugs, while 15 (25%) Candida isolates were sensitive to all antifungal drugs tested. Among 55 non-biofilm producing Candida isolates, only 7 (12.7%) were antifungal resistant isolates whereas, majority 48 (87.3%) were sensitive isolates shown in Table 5. There was statistically significant association between biofilm production and antifungal resistance (p value<0.0001).

Table 1: Antifungal susceptibility pattern of Candida species

Antifungal drug	C. albicans (n=52)		C. tropicalis (n=32)		C. glabrata (n=15)		C. krusei (n=14)		C. parapsilosis (n=2)	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
Clotrimazole	38 (73.1%)	14 (26.9%)	11 (34.4%)	21 (65.6%)	11 (73.3%)	4 (26.7%)	10 (71.4%)	4 (28.6%)	2 (100%)	00
Fluconazole	42 (80.8%)	10 (19.2%)	23 (71.9%)	9 (28.1%)	13 (86.7%)	2 (13.3%)	--	--	2 (100%)	00
Ketoconazole	43 (82.7%)	9 (17.3%)	32 (100%)	00	15 (100%)	00	--	--	2 (100%)	00
Amphotericin B	52 (100%)	00	32 (100%)	00	15 (100%)	00	14 (100%)	00	2 (100%)	00
Nystatin	52 (100%)	00	32 (100%)	00	15 (100%)	00	14 (100%)	00	2 (100%)	00

S= Sensitive R= Resistant

Table 2: Biofilm production by different Candida species

Candida species	Microtiter plate method				Visual tube method			
	Weak (%)	Moderate (%)	Strong (%)	Negative (%)	Weak (%)	Moderate (%)	Strong (%)	Negative (%)
C. albicans (n=52)	7 (13.5%)	5 (9.6%)	10 (19.2%)	30 (57.7%)	11 (21.2%)	8 (15.4%)	5 (9.6%)	28 (53.8%)
C. tropicalis (n=32)	3 (9.4%)	8 (25%)	11 (34.4%)	10 (31.2%)	4 (12.5%)	9 (28.1%)	5 (15.6%)	14 (43.8%)
C. glabrata (n=15)	1 (6.7%)	2 (13.3%)	2 (13.3%)	10 (66.7%)	3 (20%)	3 (20%)	0	9 (60%)
C. krusei (n=14)	2 (14.3%)	2 (14.3%)	7 (50%)	3 (21.4%)	4 (28.6%)	2 (14.3%)	1 (7.1%)	7 (50%)
C. parapsilosis (n=2)	0	0	0	2 (100%)	0	0	0	2 (100%)

Table 3: Biofilm production among different Candida species by Microtiter plate method

Candida species n=115	Biofilm producers (%)	Non biofilm producers (%)
Candida albicans (n=52)	22 (42.3%)	30 (57.7%)
Candida tropicalis (n=32)	22 (68.8%)	10 (31.2%)
Candida glabrata (n=15)	5 (33.3%)	10 (66.7%)
Candida krusei (n=14)	11 (78.6%)	3 (21.4%)
Candida parapsilosis (n=2)	00	2 (100%)

Table 4: Biofilm production by Non-albicans Candida and Candida albicans detected by Microtiter plate method.

n =115	Biofilm producers	Non-biofilm producers	p- value
Non-albicans Candida (n=63)	38 (60.3%)	25 (39.7%)	0.082
Candida albicans (n=52)	22 (42.3%)	30 (57.7%)	

Table 5: Correlation between biofilm production and antifungal drug resistance

n= 115	Candida isolates resistant to one or more antifungal drugs	Candida isolates sensitive to all antifungal drugs	p-value
Biofilm producers (n=60)	45 (75%)	15 (25%)	<0.0001
Non-biofilm producers (n=55)	07 (12.7%)	48 (87.3%)	

Table 6: Risk factors observed among vulvovaginal candidiasis cases

Sl. No.	Risk factors	No. of vulvovaginal candidiasis cases (n = 115)	Percentage (%)
1	Diabetes mellitus	6	5.2%
2	Hypothyroidism	5	4.3%
3	Post IUD insertion	3	2.6%
4	Chronic Kidney Disease	1	0.9%
5	Tuberculosis	1	0.9%

Table 7: Maternal and fetal outcomes among vulvovaginal candidiasis cases among biofilm producing and non-biofilm producing Candida species

Sl. No.	Maternal and fetal outcomes in ANC cases with vulvovaginal candidiasis	Biofilm producing Candida species n= 26 (%)	Non- biofilm producing Candida species n= 28 (%)
1	Emergency LSCS	5 (19.2%)	3 (10.7%)

2	Sepsis	1 (3.8%)	0 (0%)
3	Intra Uterine Growth Retardation	1 (3.8%)	0 (0%)
4	Low birth weight baby with respiratory distress	0 (0%)	1 (3.6%)

DISCUSSION

Vulvovaginal candidiasis is the common fungal infection in women of reproductive age group. Although it is common to see Candida infections in immunocompromised or chronically ill patients, its ability to survive in mucocutaneous sites, can cause infections in healthy individuals as well. Candida species are commonly isolated from genital tract in up to 25% of asymptomatic, healthy women of reproductive age. Around 70-75% of women above 25 years of age reported to have, at least 1 episode of vulvovaginal candidiasis during their lifetime and the recurrence rate is 40 - 50%.[13]

The isolation rate of Candida from vulvovaginal candidiasis cases in our study is 21.8% which is similar to a study by Makled AF et. al (15.9%).[14]

Out of 115 vulvovaginal candidiasis cases, 54 (46.9%) were antenatal care (ANC) patients and 61 (53.1%) were non-ANC patients. The various risk factors observed in our study includes diabetes mellitus seen in 6 (5.2%) cases of vulvovaginal candidiasis, hypothyroidism seen in 5 (4.3%) cases, post IUD insertion seen in 3 (2.6%) cases, chronic kidney disease and tuberculosis seen in one (0.9%) case each. [Table 6]

Candida albicans, Candida tropicalis, Candida glabrata, Candida krusei and Candida parapsilosis are the prevalent species seen in vulvovaginal candidiasis.[12,15] In our study, the above mentioned five species of Candida were isolated from cases of vulvovaginal candidiasis.

The Non-albicans Candida were more frequently isolated as compared to Candida albicans in the present study (54.8% vs 45.2%). Similar results have been seen in other Indian studies like Rudresh SM et al.(53.7% vs 46.3%),[16] Kalia N et al. (55.6% vs 44.4%),[17] Gupta S et al.(63.7% vs 36.3%).[18] Among Non-albicans Candida species, Candida tropicalis was the most frequently isolated (27.8%), followed by Candida glabrata (13.1%), Candida krusei (12.2%) and Candida parapsilosis (1.7%). The distribution of Candida species is similar to study by Maheshwari P et al.[19] This study was done on pregnant women attending a tertiary care hospital in Indore, where the isolation rate of Candida albicans is 51.7% followed by Candida tropicalis (28.3%), Candida krusei (10%), Candida glabrata (8.3%) and Candida parapsilosis (5%). Prolonged treatment for recurrent candidiasis, widespread use of over-the-counter antifungal drugs, non-compliance to the treatment and use of effective antifungal agents to eliminate Candida albicans are possible explanations for the increased isolation of Non-albicans Candida species from vulvovaginitis patients.[20]

In our hospital, clotrimazole given as pessary is the most commonly used antifungal drug followed by fluconazole to treat vulvovaginal candidiasis. However, 46.1% of Non-albicans Candida isolates were resistant to clotrimazole and 17.5% were resistant to fluconazole. 26.9% and 19.2% of Candida albicans isolates were resistant to clotrimazole and fluconazole respectively, which could be related to widespread use of these drugs. No resistance was observed for amphotericin B and nystatin among all the Candida species in our study.

The antifungal resistance pattern seen in our study correlates with the study done by Khan N et al.[13] They observed that, among Non-albicans Candida, 46% were resistant to clotrimazole, 36.8% were resistant to fluconazole, 6.6% were resistant to nystatin, 5% were resistant to ketoconazole and 2.6% were resistant to amphotericin B. Among Candida albicans, 21.6% isolates were resistant to clotrimazole, 13.5% were resistant to fluconazole, 2.8% were resistant to ketoconazole. No resistance was observed among Candida albicans for amphotericin B and nystatin.

A biofilm is a community of microorganisms and their extra cellular polymers that are attached to a surface. Biofilm producing microorganisms are responsible for many recalcitrant infections and are difficult to eradicate. They exhibit resistance to antifungals by various methods like restricted penetration of drugs, decreased growth rate and expression of resistance genes.[21] Biofilm development also weakens the host immune response by hindering macrophage phagocytosis and antibody activity.[22]

Biofilm-associated infections are becoming more common in clinical settings, accounting for 65% to 80% of all infections. Although the majority of studies have focused on Candida albicans in the biofilm formation, several authors have found that Non-albicans Candida are also involved in biofilm development.[23]

Literature suggests that the Microtiter plate method is the gold standard method for biofilm detection.[24] As per the results of Microtiter plate method, we observed that 52.2% of Candida isolates were biofilm producers which is comparable to a study by Tulasidas S et al,[1] and Mohammadi F et al,[5] where 44.1% and 54.3% of the Candida isolates from cervical swabs were biofilm producers respectively.

It was also observed in our study that; biofilm production was common among Non-albicans Candida (60.3%) as compared to Candida albicans (42.3%). The results of our study are comparable with the other studies like Marak MB et al,[8] and Das KH et al,[25] where the biofilm production was 57.14% & 87.5% among Non-albicans Candida and 39.02% and 75% among Candida albicans respectively. Hence, Non-albicans Candida cannot be ignored in vulvovaginal candidiasis cases because of their ability to produce biofilm which in

turn increases resistance to antifungal drugs, making it difficult to treat these infections.[1]

In the present study, 42.3% of Candida albicans exhibited biofilm production which is similar to the results seen in Alikhani T et al,[26] where 48.7% of the Candida albicans were positive for biofilm production.

Our study observed that the resistance to antifungal drugs was common among biofilm producers (75%) as compared to non-biofilm producers (12.7%). Similar trend was also seen in a study by Moursi SA et al,[27] where, 81.7% of Candida isolates were resistant to antifungal drugs among biofilm producers whereas, 18.3% were resistant among non-biofilm producers.

In our study, we observed adverse maternal and fetal outcomes among ANC cases like emergency LSCS seen in 5 (19.2%) isolates of biofilm producing Candida species, sepsis and IUGR seen in one (3.8%) isolate each of biofilm producing species. Among non-biofilm producing Candida species, 10.7% had outcomes like emergency LSCS and 3.6% had low birth weight baby with respiratory distress [Table 7].

The present study highlights that there is an increase in the isolation of Non- albicans Candida species. Our study clearly shows increased antifungal resistance in Non - albicans Candida species. In addition, our study demonstrated that biofilm formation was associated with increased antifungal resistance.

Therefore, the findings of our study reiterate the importance of species level identification for Candida isolates, timely done antifungal susceptibility testing and assessment of biofilm forming ability. This information will help the clinician to choose appropriate antifungal therapy and help limit the emergence of antifungal resistance.

CONCLUSION

Vulvovaginal candidiasis causes inflammation and pruritis in women. The etiological agent of vulvovaginal candidiasis in our study belong to Non-albicans Candida group, predominantly Candida tropicalis. Antifungal resistance was observed to clotrimazole and fluconazole. However, antifungal resistance was more in Non-albicans Candida group. Majority of Non-albicans Candida were biofilm producers. There was a significant association between biofilm production and antifungal resistance. Therefore, this study concludes that prompt identification of Candida species along with antifungal susceptibility testing and biofilm production is required to initiate appropriate and timely antifungal therapy for better patient outcome and to decrease antifungal resistance.

Declaration statements

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Data availability: All data associated with this study is included in the manuscript.

Ethics statement: Institutional Ethics Committee approval was obtained for the study. (Ref: BGSGIMS/IEC/App/Sep/2024/08 dated 28th September 2024).

Informed consent: All the samples were included in the study after obtaining the informed consent from the patients.

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